Annals of Clinical and Analytical Medicine
Original Research

Evaluation of the relationship between disease severity and the leptin, adiponectin, and chemerin levels in overweight and obese COVID-19 patients

Leptin, adiponectin and chemerin levels in COVID-19 severity

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Abstract

Aim: Overweight and obesity are substantial risk factors in the severity of COVID-19 disease. This study aimed to assess the relationship between the disease severity and leptin, adiponectin, and chemerin levels in overweight and obese COVID-19 patients.

Material and Methods: The study involved 60 COVID-19 patients (patient group) and 30 healthy controls with BMI≥25. The patient group was split into two subgroups based on disease severity (30 mild/moderate and 30 severe/critical patients). The levels of leptin, adiponectin, and chemerin in plasma were determined using the ELISA technique.

Results: Our study revealed that leptin levels were considerably increased in both groups of COVID-19 patients compared to the healthy controls, while chemerin levels were decreased. In adiponectin levels, there was no statistically significant difference between the groups.

Discussion: Plasma leptin and chemerin levels are associated with the progression and/or severity of disease in overweight and obese COVID-19 patients.

Keywords

COVID-19, Leptin, Adiponectin, Chemerin, Obesity

DOI: 10.4328/ACAM.22078 Received: 2023-12-19 Accepted: 2024-02-12 Published Online: 2024-03-22 Printed: 2024-05-01 Ann Clin Anal Med 2024;15(5):329-333
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This study was approved by the Ethics Committee of Nigde Omer Halisdemir University (Date: 2021-02-21, No: 23)

Introduction

Coronavirus disease 2019 (COVID-19) is an exceptionally complex disease with a wide variety of clinical manifestations ranging from mild to severe and critical disease [1]. Although the mechanisms underlying the pathophysiology of COVID-19 disease are not yet fully understood, overweight and obesity are acknowledged to considerably contribute to the disease's severity through a variety of processes. Notably, recent studies have revealed that overweight and obesity are substantial risk factors for hospitalization, intensive care unit (ICU) admission, respiratory failure, and invasive mechanical ventilation (IMV) requirements in COVID-19 patients [2-4].

Obesity represents a state of low-grade inflammation because of the chemoattraction of macrophages and its expansion in the adipose tissue [5, 6]. Adipose tissue secretes a large number of molecules known as adipokines, which are involved in the regulation of many pivotal biological processes, including inflammation and immunity [7]. Dysregulated adipokines synthesis from adipose tissue may contribute to the emergence of the "cytokine storm" that characterized the severe form of COVID-19 and can be presented as a plausible mechanism explaining the influence of obesity on disease severity [5, 6, 8].

Leptin is a pleiotropic hormone, mainly released by white adipose tissue which has significant functions in energy regulation, endocrine, metabolism, and inflammation. In addition to these functions, leptin is a pro-inflammatory adipokine that promotes both innate and adaptive immune responses [3, 4]. Adiponectin is the most prevalent plasma protein and exhibits anti-inflammatory properties. It possesses antioxidant, anti-fibrotic, anti-atherogenic, and anti-apoptotic properties and is engaged in a range of biological processes such as insulin sensitization, glucose regulation, and fatty acid oxidation. It has been reported that adiponectin levels were decreased in metabolic disorders such as diabetes and obesity [9]. Chemerin is a novel adipokine with autocrine, paracrine, and endocrine effects. It modulates energy metabolism, angiogenesis, and adipogenesis, as well as innate and adaptive immunity by functioning as a powerful chemoattractant protein for immune cells. It has also been demonstrated to increase considerably in several inflammatory and metabolic diseases [10, 11].

Given that background, these adipokines may be involved in the relationship between the heightened inflammatory response and dysfunctional adipose tissue in severe COVID-19 disease, albeit their role in the disease is not entirely known. There are few data in the literature on the relationship between the severity of disease and plasma leptin, adiponectin, and chemerin levels, and the findings are contradictory with each other. Hence, the purpose of this study was to evaluate the relationship between the severity of the disease and levels of leptin, adiponectin, and chemerin in overweight and obese COVID-19 patients.

Material and Methods

Study population

This prospective study consisted of 60 patients with RT-PCR-confirmed diagnosis of COVID-19 (patient group) and 30 healthy control (control group), who applied to the Clinic of Infectious Disease, Internal Medicine, and Intensive Care Unit of Kayseri City Hospital, Turkey. The patient group was split into two groups based on the severity of the disease: mild/moderate (n:30) and severe/critical (n:30) [1]. The study inclusion criteria for all the groups were: (1) age ≥ 18 years, and (2) Body Mass Index (BMI) ≥25. The study exclusion criteria for all the groups were: (1) the presence of chronic systemic inflammatory disease, (2) the presence of metabolic disease, (3) the presence of chronic kidney and liver failure, and (4) the presence of malignancy. Demographic characteristics and laboratory parameters of all participants were retrieved from the patient files by the physicians who followed the patients. Laboratory parameters were measured using routine laboratory techniques within 24 hours of admission. BMI was divided into six categories according to WHO classification [12].

Collection of blood samples

5 mL of blood was collected from each participant into EDTA-containing tubes and centrifuged at 1500 g for 10 minutes at 4°C. The plasma samples were kept in aliquots at -80 °C until the enzyme-linked immunosorbent assay (ELISA) analysis was performed.

Plasma leptin, adiponectin, and chemerin analyses

Leptin (USCN, SEA084Hu), adiponectin (USCN, SEA605Hu), and chemerin (USCN, SEA945Hu) levels in plasma were determined using an ELISA kit according to the manufacturer's instructions. All samples were diluted 1/10, and absorbance values were recorded by reading at 450 nm with a BioTek ELx800 absorbance microplate reader.

Statistics Analyses

Statistical analyses were carried out by using the IBM SPSS Statistics version 23 (IBM Corp, Armonk, NY, USA). Categorical variables were presented as percentages (%) and/or numbers (n). The normality of the distribution of the data was determined with the Shapiro–Wilk test. Data were expressed as mean ± standard deviation (SD) and median [interquartile range (IQR; 25%-75%)] for variables with normal and non-normal distributions, respectively. In the comparison of data between groups, the ANOVA test, followed by the post-hoc test was used for data with normal distribution, and the Kruskal Wallis test, followed by the Mann-Whitney U test was used for non-normal distribution data. The level of statistical significance was defined as $p<0.05$.

Ethical Approval

This study was approved by the Ethics Committee of Nigde Omer Halisdemir University (Date: 2021-02-21, No: 23).

Results

As shown in Table 1, in the control group, there were 14 (46.7%) males and 16 (53.3%) females; in the mild/moderate patient group, there were 14 (46.7%) males and 16 (53.3%) females; and in the severe/critical group, there were 12 (40%) males and 18 (60%) females. There was no statistically significant difference between the groups in terms of gender (p=0.892), age (p=0.132), and BMI (0.245).

WBC and neutrophil counts were significantly higher in the severe/critical patient group than in the control and the mild/moderate patient group. Lymphocyte levels were significantly higher in the mild/moderate group compared to the control group, and lower in the severe/critical patient group than in the mild/moderate patient group. CRP and AST levels in both

Table 1. Demographic and routine laboratory characteristics of the study groups

Parameters	Control Group (n:30)	Patient Groups (n:60) Mild/moderate group (n:30)	Severe/critical group (n:30)	p value
Gender (M/F)	14/16 (46.7%/53.3%)	14/16 (46.7%/53.3%)	12/18 (40%/60%)	0.892
Age, years	49.75±13.10	45.48±12.05	55.14±16.20	0.132
BMI, kg/m2	28.25 (26.70-31.50)	29.30 (28.03-33.10)	31.0 (27.0-33.0)	0.245
WBC,103/µL	6.11 (4.60-7.42)	5.62 (5.15-8.22)	9.90 (5.04-12.30)*,a	0.042
Neutrophil,103/µL	3.64±2.00	4.41±1.95	7.50±3.32*,a	0.001
Lymphocyte, 103/µL	1.04 (0.12-1.69)	1.49 (1.20-1.92)*	0.98 (0.65-1.37)a	0.001
CRP, mg/L	2.08 (1.03-3.71)	11.00 (3.62-22.58)*	12.70 (4.67-56.05)*	0.000
PLT, 103/µL	261.0 (243.0-304.0)	249.0(198.5-306.0)	236.5 (219.75-314.75)	0.732
Prokalsitonin, µg/L	0.04 (0.03-0.08)	0.06 (0.03-0.15)	0.07 (0.06-0.11)	0.087
D-Dimer, FEU, µg/L	356 (312.5-431.0)	390 (275-580)	510.0 (326.75-780.0)	0.082
LDH, U/L	257.5 (233.5-330)	227.0 (169.5-270.5)*	300.0 (243.25-359.0)a	0.005
ALT, U/L	17.75 (15.00-23.02)	21.00 (16.00-43.95)	35.00(24.25-42.00)*	0.003
AST, U/L	17.50 (13.50-23.52)	22 (19-40)*	30 (22-39)*	0.001
BUN, mg/dL	15.05 (12.45-19.93)	12.3 (10.1-16.2)*	17.00 (15.25-24.25)a	0.002
Creatinine, mg/dL	0.79 (0.69-0.95)	0.81 (0.65-0.94)	0.77 (0.65-0.96)	0.709

Data were expressed as mean ± standard deviation or median (25%-75% quartiles). ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: Blood Urea Nitrogen; CRP: C-reactive protein; LDH: lactate dehydrogenase; WBC: white blood cell; PLT: platelet. *: Differences compared to the control group; a: Differences between mild/moderate group and severe/critical group.

Table 2. Plasma levels of leptin, adiponectin, and chemerin

	Control Group (n:30)	Patient Groups (n:60) Mild/moderate group (n:30)	Severe/critical group (n:30)	p value
Leptin (ng/mL)	5.83 (5.21-7.67)	7.75 (6.55-9.37)*	9.05 (8.27-9.84)*, [a]	0.001
Adiponectin (ng/mL)	9.06 (8.54-9.26)	9.02 (8.52-9.42)	9.03 (8.50-9.51)	0.592
Chemerin (ng/mL)	89.04±15.94	61.11±12.61*	53.19±14.45*	0.001

Data were expressed as mean ± standard deviation or median (25%-75% quartiles). *: Differences compared to the control group; a: Differences between mild/moderate group and severe/critical group.

patient groups were found to be significantly higher than in the control group. ALT levels were considerably higher in the severe/critical patient group than in the control group. LDH and BUN levels were significantly lower in the mild/moderate group compared to the control group, and higher in the severe/critical patient group than in the mild/moderate patient group (Table 1).

Plasma leptin levels were significantly increased in both COVID-19 patient groups compared to the control group, with the highest levels found in severe/critical patients. There was no statistically significant difference in plasma adiponectin levels between the groups. Chemerin levels were considerably decreased in both patient groups compared to the control group; however, there was no statistically significant difference between the patient groups (Table 2).

Discussion

Adipokines are bioactive molecules that have pleiotropic effects. In recent years, a number of studies have revealed that they play a crucial role in the development of several diseases and in regulating metabolism, inflammation, and immunity. On the other hand, adipokine dysregulation leads to obesity-related diseases. Especially, since the majority of adipokines are elevated in obese individuals and contribute to low-grade inflammation, they are now regarded as important players in inflammation and immunity [7]. However, their role on the development and severity of COVID-19 disease is not yet fully known. Therefore, in this study, we evaluated the relationship between disease severity and leptin, adiponectin, and chemerin levels in COVID-19 patients with overweight and obese.

Leptin, as a pro-inflammatory cytokine, may serve as a link between obesity, metabolism, and inflammatory diseases [4]. Leptin levels reflect the amount of energy stored and were positively associated with fat mass and BMI [7]. Nevertheless, the study results regarding leptin levels in COVID-19 disease are confusing, and it has been reported that COVID-19 patients had increased or decreased leptin levels [13-16]. In our study, we found that plasma leptin levels increased in both patient groups compared to the control group, and their levels increased with the enhanced severity of the disease. Consistent with our results, Wang et al. demonstrated that as compared to healthy controls and mild patients, leptin levels in severe COVID-19 patients are considerably higher and that these increased levels are associated with systemic inflammation, disease severity, and progression. They have also shown that in patients with a BMI >24, the increase of leptin was greater in severe patients compared to mild patients. Additionally, COVID-19 patients with overweight had higher leptin levels, which further activated monocytes, and led to dysregulated or amplified immune responses [13]. Tonon et al. have indicated that patients with COVID-19 pneumonia had increased leptin levels as compared to healthy controls. In addition, leptin has an acceptable discriminatory accuracy for COVID-19 pneumonia in patients with BMI>30 and was related to maximum respiratory support [14]. Similar findings were obtained by Van der Voort et al. who reported that COVID-19 patients had significantly

higher levels of serum leptin compared to control patients. Additionally, they also suggested that excessive adipose tissue and elevated levels of leptin in COVID-19 patients can trigger the development of respiratory failure and acute respiratory distress syndrome [15]. All of these findings suggest that increased leptin levels following infection may play a pivotal role in the mechanisms leading to the severe progression of COVID-19, particularly in obese and overweight individuals [5, 13, 15, 16]. On the other hand, contrary to our findings, few studies have also reported that there is no association between leptin and disease severity in COVID-19 [17-19]. Di Filippo et al. reported that there was no significant difference in leptin levels of patients with severe COVID-19 compared to the patients with mild and moderate COVID-19. However, they attributed the discrepancy with the findings of other studies to the fact that they measured leptin shortly after hospitalization, and also noted that leptin levels may increase later in those with an insufficient anti-inflammatory response at the onset of the disease [17].

Adiponectin is an adipokine that has anti-inflammatory properties and it has been shown in various studies that decreased adiponectin levels are associated with metabolic syndrome, obesity, and inflammation [8, 9]. The role of adiponectin in COVID-19 disease is still unclear, and reviewing literature showed that the results of the previous studies on COVID-19 contradict each other [17-21]. Reiterer et al. found that adiponectin levels had significantly decreased in severe COVID-19 patients [20]. Likewise, Kearns et al. reported that patients with COVID-19 ICU with acute respiratory failure were related to decreased adiponectin levels even after adjusting BMI and these decreased adiponectin levels played an essential role in the association between obesity and COVID-19. Furthermore, they also implied that hypoadiponectinemia, which is common in obese patients, may facilitate the increased inflammatory response to the pulmonary capillaries [21]. In contrast to the results described above, we found that there was no significant difference between the groups in terms of adiponectin levels. Similarly, one study conducted by Minuzzi et al. demonstrated that adiponectin levels were not associated with IC requirement or outcome in obese COVID-19 patients [19]. Additionally, it has been reported that there is no relationship between adiponectin levels and disease severity [17, 18]. These observed discrepancies may be due to differences in the patient cohort.

Chemerin is an intriguing adipokine that has gained recognition as a metabolic and immunological process regulator; bridging inflammatory diseases and obesity and its levels are considerably increased in these diseases. Furthermore, it has been also reported that chemerin levels are positively correlated with several inflammatory markers and obesity-related markers [10, 11]. There have been few studies on the levels of chemerin in COVID-19. Nonetheless, like leptin and adiponectin, results regarding levels of chemerin remain debatable [8, 22-25]. Lavis et al. found that plasma chemerin levels were significantly higher in COVID-19 patients compared to healthy controls and correlated with inflammation and disease severity [22]. Similar results have been reported by Hussein et al [23]. Fioravanti et al. implied that tocilizumab, an IL-6 receptor antagonist, can be used in reducing chemerin circulating levels and treatment of severe complications in COVID-19 patients, particularly in obesity, by modulating it [8]. In contrast to these results, our investigation indicated that chemerin levels were significantly decreased in both groups of COVID-19 patients compared to the healthy controls, but were not associated with disease severity. This result is one of the remarkable findings of the present study. In line with our findings, Kukla et al. demonstrated that chemerin levels were considerably decreased in COVID-19 patients compared to healthy controls. They also found no relationship between disease severity and decreased chemerin levels [24]. Sulicka-Grodzicka et al. found that chemerin levels decreased one week following the onset of symptoms in moderate and severe COVID-19 patients and that this decline may be related to an enhanced inflammatory response in patients with more severe infections [25]. These findings do not appear to be compatible with previous research indicating that chemerin has pro-inflammatory properties. Nonetheless, although chemerin is generally known as a pro-inflammatory adipokine, experimental research has revealed that it exhibits pro- or anti-inflammatory effects that vary depending on the tissue and stimulus in which it is activated and various clinical circumstances [10, 11, 22]. Taking into consideration our results, in this study, it was shown that chemerin may also have anti-inflammatory effects and play a role in the pathogenesis of COVID-19. Further studies are needed to determine the role of chemerin.

Conclusion

In conclusion, increased leptin and decreased chemerin levels have been associated with the progression and/or severity of disease in overweight and obese COVID-19 patients. These results support the hypothesis that leptin and chemerin levels play a significant role in the inflammatory process and the mechanisms underlying the exacerbation of COVID-19 after infection in overweight and obese patients. Furthermore, although chemerin is generally known as a pro-inflammatory adipokine, this study also showed that it may possess anti-inflammatory effects.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

Funding: *This work was supported by the Nigde Omer Halisdemir University Scientific Research Projects Coordination Unit under Project number: SAT 2022/3-BAGEP.*

Conflict of Interest

The authors declare that there is no conflict of interest.

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How to cite this article:
Gonul Seyda Seydel, Inayet Gunturk, Cevat Yazıcı, Ulas Serkan Topaloglu, Esma Eryılmaz Eren, Recep Civan Yuksel. *Evaluation of the relationship between disease severity and the leptin, adiponectin, and chemerin levels in overweight and obese COVID-19 patients. Ann Clin Anal Med 2024;15(5):329-333*

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